JC03 Rec'd PCT/PTO 30 MAR 2001

09/806437

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
REQUEST FOR FILING NATIONAL PHASE OF
PCT APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

To: Hon. Commissioner of Patents
Washington, D.C. 20231

00909

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

Atty Dkt: P 279277 /2892USAS/VO
M# /Client Ref.

From: Pillsbury Winthrop LLP, IP Group:

Date: March 30, 2001

This is a **REQUEST** for **FILING** a PCT/USA National Phase Application based on:

1. International Application	2. International Filing Date	3. Earliest Priority Date Claimed
PCT/EP99/07692	13 October 1999	15 October 1998
⇧ country code	Day MONTH Year	Day MONTH Year (use item 2 if no earlier priority)

4. Measured from the earliest priority date in item 3, this PCT/USA National Phase Application Request is being filed within:

(a) ☐ 20 months from above item 3 date (b) ☒ 30 months from above item 3 date,

(c) Therefore, the due date (unextendable) is April 15, 2001

5. Title of Invention METHOD AND SUBSTANCES FOR DIAGNOSIS AND THERAPY OF SEPSIS AND SEPSIS-LIKE SYSTEMIC INFECTIONS

6. Inventor(s) BERGMANN, Andreas et al

Applicant herewith submits the following under 35 U.S.C. 371 to effect filing:

7. ☒ Please immediately start national examination procedures (35 U.S.C. 371 (f)).

8. ☐ **A copy of the International Application** as filed (35 U.S.C. 371(c)(2)) is transmitted herewith (file if in English but, if in foreign language, file only if not transmitted to PTO by the International Bureau) including:

a. ☐ Request;
b. ☐ Abstract;
c. _____ pgs. Spec. and Claims;
d. _____ sheet(s) Drawing which are ☐ informal ☐ formal of size ☐ A4 ☐ 11"

9. ☒ **A copy of the International Application has been transmitted by the International Bureau.**

10. **A translation of the International Application** into English (35 U.S.C. 371(c)(2))
a. ☒ is transmitted herewith including: (1) ☐ Request; (2) ☒ Abstract;
(3) 24 pgs. Spec. and Claims;
(4) 10 sheet(s) Drawing which are:
☐ informal ☒ formal of size ☒ A4 ☐ 11"
b. ☐ is not required, as the application was filed in English.
c. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.
d. ☐ Translation verification attached (not required now).

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11. ☒ Please see the attached Preliminary Amendment

12. ☐ Amendments to the claims of the International Application **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., before 18th month from first priority date above in item 3, are transmitted herewith (file only if in English) including:**

13. ☒ PCT Article 19 claim amendments (if any) have been transmitted by the International Bureau

14. ☐ Translation of the amendments to the claims **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., of claim amendments** made before 18th month, **is attached (required by 20th month from the date in item 3 if box 4(a) above is X'd, or 30th month if box 4(b) is X'd, or else amendments will be considered canceled).**

15. **A declaration of the inventor** (35 U.S.C. 371(c)(4))
a. ☐ is submitted herewith ☐ Original ☐ Facsimile/Copy
b. ☒ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

16. **An International Search Report (ISR):**
a. Was prepared by ☒ European Patent Office ☐ Japanese Patent Office ☐ Other
b. ☒ has been transmitted by the international Bureau to PTO.
c. ☒ copy herewith (5 pg(s).) ☒ plus Annex of family members (1 pg(s).).

17. **International Preliminary Examination Report** (IPER):
a. ☒ has been transmitted (if this letter is filed after 28 months from date in item 3) in English by the International Bureau with Annexes (if any) in original language.
b. ☐ copy herewith in English.
c.1 ☐ IPER Annex(es) in original language ("Annexes" are amendments made to claims/spec/drawings during Examination) including attached amended:
c.2 ☐ Specification/claim pages #_____ claims #
Dwg Sheets #
d. ☐ Translation of Annex(es) to IPER **(required by 30th month due date, or else annexed amendments will be considered canceled).**

18. **Information Disclosure Statement** including:
a. ☒ Attached Form PTO-1449 listing documents
b. ☒ Attached copies of documents listed on Form PTO-1449
c. ☒ A concise explanation of relevance of ISR references is given in the ISR.

19. ☐ **Assignment** document and Cover Sheet for recording are attached. Please mail the recorded assignment document back to the person whose signature, name and address appear at the end of this letter.

20. ☐ Copy of Power to IA agent.

21. ☐ **Drawings** (complete only if 8d or 10a(4) not completed): ___ sheet(s) per set: ☐ 1 set informal; ☐ Formal of size ☐ A4 ☐ 11"

22. Small Entity Status ☐ is **Not** claimed ☒ is claimed (**pre**-filing confirmation required)
22(a) _____ (No.) Small Entity Statement(s) enclosed (since 9/8/00 Small Entity Statements(s) not essential to make claim)

23. **Priority** is hereby claimed under 35 U.S.C. 119/365 based on the priority claim and the certified copy, both filed in the International Application during the international stage based on the filing in (country) GERMANY of:

	Application No.	Filing Date		Application No.	Filing Date
(1)	198 47 690.6	October 15, 1998	(2)		
(3)			(4)		
(5)			(6)		

a. ☒ See Form PCT/IB/304 sent to US/DO with copy of priority documents. If copy has not been received, please proceed promptly to obtain same from the IB.
b. ☐ Copy of Form PCT/IB/304 attached.

RE: USA National Phase Filing of PCT/EP99/07692

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24. Attached:

25 Per Item 17.c2, **cancel original** pages #_____, claims #_____, Drawing Sheets #

26. **Calculation of the U.S. National Fee (35 U.S.C. 371 (c)(1)) and other fees is as follows:**
Based on amended claim(s) per above item(s) ☐ 12, ☐ 14, ☐ 17, ☐ 25 (hilite)

Total Effective Claims	minus 20 =	x $18/$9 =	$0	966/967
Independent Claims	minus 3 =	x $80/$40 =	$0	964/965
If any proper (ignore improper) Multiple Dependent claim is present,		add$270/$135	+0	968/969

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): →→ **BASIC FEE REQUIRED, NOW** →→→→

A. If country code letters in item 1 are **not** "US","BR","BB","TT","MX","IL" "NZ", "IN" or "ZA"

See item 16 re:

1.	Search Report was not prepared by EPO or JPO - - - - - - - - -	add$1000/$500		960/961
2.	Search Report was prepared by EPO or JPO - - - - - - - - - - -	add$860/$430	+430	970/971

SKIP B, C, D AND E UNLESS country code letters in item 1 are "US","BR","BB","TT","MX","IL", "NZ", "IN" or "ZA"

☒ (X) →	☐ B. If USPTO did not issue both International Search Report (ISR) and (if box 4(b) above is X'd) the International Examination Report (IPER), - - - - - - - - - - - - - - - - - - -	add$970/$485	+0	960/961
☐ (only) (one) →	☐ C. If USPTO issued ISR but not IPER (or box 4(a) above is X'd), -	add$710/$355	+0	958/959
(of) (these) (4) →	☐ D. If USPTO issued IPER but IPER Sec. V boxes not all 3 YES, -	add$690/$345	+0	956/957
☐ →	☐ E. If international preliminary examination fee was paid to USPTO and Rules 492(a)(4) and 496(b) satisfied (IPER Sec. V all 3 boxes YES for all claims), - - - - - - - - - - - - -	add $100/$50	+0	962/963
(boxes)		**SUBTOTAL =**	$430	

27.

28. If Assignment box 19 above is X'd, add Assignment Recording fee of ----$40 +0 (581)

29. Attached is a check to cover the - **TOTAL FEES** $430

Our Deposit Account No. 03-3975
Our Order No. 11377 (C#) | 279277 (M#)

00909

CHARGE STATEMENT: The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 and 492 (missing or insufficient fee only) now or hereafter relative to this application and the resulting Official document under Rule 20, or credit any overpayment, to our Account/Order Nos. shown above for which purpose a duplicate copy of this sheet is attached.
This CHARGE STATEMENT does not authorize charge of the issue fee until/unless an issue fee transmittal form is filed

Pillsbury Winthrop LLP
Intellectual Property Group

By Atty: Paul N. Kokulis **Reg. No.** 16773

Sig:

Fax: (202) 822-0944
Tel: (202) 861-3503

Atty/Sec: PNK/mhn

NOTE: File in duplicate with 2 postcard receipts (PAT-103) & attachments.

Inventor(s): BERGMANN ET AL (Atty. Dkt.

Appln. No.: 09/806,437 or Patent No.: 279277/2892 USAS/VO

Filed: March 30, 2001 or Issued.: M# / Client Ref.

Title: METHOD AND SUBSTANCES FOR DIAGNOSIS AND THERAPY OF SEPSIS AND SEPSIS-LIKE SYSTEMIC INFECTIONS

SMALL ENTITY STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(d) and 1.27 (c)) - **SMALL BUSINESS CONCERN**

I hereby state that I am

☐ the owner of the small business concern identified below:

☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN B.R.A.H.M.S DIAGNOSTICA GMBH

ADDRESS OF CONCERN Neuendorfstrasse 25, 16761 Hennigsdorf, Germany

I hereby state that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby state that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention **entitled:** METHOD AND SUBSTANCES FOR DIAGNOSIS AND THERAPY OF SEPSIS AND SEPSIS-LIKE SYSTEMIC INFECTIONS
by inventor(s) Bergmann et al described in

box x → ☐ the specification filed herewith,
box one → ☒ Application No. 09/806,437, filed March 30, 2001
box two → ☐ Patent No. _____, issued

If the rights held by the above identified small business concern are not exclusive, each small entity individual, concern or organization having rights to the invention is listed in (A) and (B) below and no rights to the invention are held by any person, other than the inventor, who could not qualify under 37 CFR 1.9(c) as an independent inventor if that person had made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1 9(d) or a nonprofit organization under 37 CFR 1.9(e).

(A) FULL NAME of assignee/licensee/grantee/conveyee*
ADDRESS
☐ INDIVIDUAL ☒ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

(B) FULL NAME of assignee/licensee/grantee/conveyee*
ADDRESS
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

*NOTE: Separate statement is required from each person, concern or organization named in (A) and (B) above having rights to the invention, averring to his/her/its status as a small entity. (37 CFR 1.27)

I acknowledge the duty to file, in this case, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

NAME OF PERSON SIGNING Dr. Andreas Bergmann
TITLE OF PERSON OTHER THAN OWNER Vice President
ADDRESS OF PERSON SIGNING c/o BRAHMS Diagnostica, Neuendorfstr. 25, 16761 Hennigsdorf

SIGNATURE _____ DATE April 20, 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

Inventor(s): BERGMANN, Andreas et al

Filed: Herewith

Title: METHOD AND SUBSTANCES FOR DIAGNOSIS AND THERAPY OF SEPSIS AND SEPSIS-LIKE SYSTEMIC INFECTIONS

March 30, 2001

PRELIMINARY AMENDMENT

Hon. Commissioner of Patents
Washington, D.C. 20231

Sir:

Please amend this application as follows:

IN THE SPECIFICATION:

At the top of the first page, just under the title, insert

☒ --This application is the National Phase of International Application PCT/EP99/07692 filed October 13, 1999 which designated the U.S. and that International Application

☐ was ☒ was not published under PCT Article 21(2) in English.--

Respectfully submitted,

PILLSBURY WINTHROP LLP
Intellectual Property Group

By: _____

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Methods and substances for the diagnosis and therapy of sepsis and sepsis-like systemic infections

The present invention relates to novel diagnostic and therapeutic possibilities which could be derived from novel, experimentally confirmed discoveries in connection with the occurrence of procalcitonin or procalcitonin partial peptides in sepsis and severe sepsis-like systemic infections.

The patents DE 42 27 454 and EP 0 656 121 B1 and US 5,639,617 disclose that the determination of the prohormone procalcitonin and of partial peptides derived therefrom in a serum or plasma of a patient in whom there is a risk of sepsis and in whom symptoms typical of sepsis are found is a valuable diagnostic aid for early detection, i.e. for the detection of infections which may lead to sepsis, and their differentiation from noninfectious etiologies, for the detection of the severity and for the assessment of the success of a treatment of sepsis and sepsis-like systemic infections. Said determination has proved particularly valuable for

diagnosis to distinguish symptoms attributable to systemic microbial infections from other symptoms of noninfectious etiology which, owing to their clinical picture, might suggest a sepsis but in reality are not attributable to a systemic microbial infection, for example from symptoms attributable to noninfectious inflammations of individual organs, to postoperative rejection reactions or cancers. Furthermore, systemic inflammations can be distinguished from local ones.

For an overview of the more recent discoveries, reference is made to W. Karzai et al. in Infection, Vol. 25 (1997), 6, pages 329-334 and the further technical literature cited or mentioned therein.

Procalcitonin became known as a prohormone of calcitonin, and its complete amino acid sequence has long been known (FEBS 167 (1984), page 93-97). Procalcitonin is produced under normal conditions in the C cells of the thyroid gland and then specifically cleaved into the hormone calcitonin and the further partial peptides katacalcin and an N-terminal residue comprising 57 amino acids ("aminoprocalcitonin").

Since in the case of sepsis greatly elevated procalcitonin levels are observed even in patients from whom the thyroid gland was completely removed, it was necessary to conclude that the procalcitonin detectable in the blood of sepsis patients is formed outside the thyroid gland, different opinions having been expressed in the technical literature, some of them supported by experimental material, with regard to the organs or cells or the tissues which are critical for procalcitonin production during sepsis.

Regarding the nature of the peptide determined as "procalcitonin" in sepsis, it was in fact made clear from

the outset in the above-mentioned patients that the specific peptide need not be completely identical to the known procalcitonin peptide of full length, which is formed in the thyroid glands as a calcitonin precursor. However, the question as to whether the procalcitonin formed in the case of sepsis differs from the procalcitonin formed in the thyroid glands remain unanswered to date. Possible differences were posttranslational modifications of the known procalcitonin, such as glycosylations, phosphorylations or modifications of the primary structure, but also modified, shortened or lengthened amino acid sequences. Since the analytical assay methods used to date did not reveal any differences between the procalcitonin known as the calcitonin precursor and the procalcitonin formed in the case of sepsis, it was provisionally generally assumed that the procalcitonin formed in the case of sepsis is identical to the calcitonin precursor and is thus a peptide having the known procalcitonin sequence of 116 amino acids (procalcitonin 1-116).

As revealed by the determinations in the Applicant's laboratory, explained in more detail in the experimental section of this Application, however, the procalcitonin formed in the case of sepsis differs slightly but significantly from the complete procalcitonin 1-116 formed in the thyroid gland. The differences found then led to a number of scientific conclusions which could be implemented in novel diagnostic and therapeutic methods, substances usable therein and scientific approaches which could be pursued.

The starting point for the invention disclosed in the present Patent Application is the surprising discovery that the procalcitonin detectable in comparatively high concentrations in the serum of patients in the case of sepsis and sepsis-like systemic infection is not the

complete procalcitonin 1-116 comprising 116 amino acids but procalcitonin shortened at the amino terminus by a dipeptide but otherwise identical and having an amino acid sequence of only 114 amino acids (procalcitonin 3-116).

The dipeptide missing in comparison with the complete procalcitonin has the structure Ala-Pro. The lack of a dipeptide comprising a proline residue as a second amino acid of the amino terminus of the complete procalcitonin sequence led to the presumption that a specific peptidase might play a role in the production of the procalcitonin 3-116 detectable in the case of sepsis, that is to say the so-called dipeptidyl-(amino)-peptidase IV (DP IV or DAP IV or CD26).

For the determination of a possible role of the dipeptidyl-aminopeptidase IV in association with systemic infection or with sepsis, the inventors have therefore tested experimentally whether a correlation of the physiological DAP IV concentrations with the detection of a sepsis is possible. The results obtained showed such a correlation.

The more exact results obtained furthermore led to the development of a hypothesis that the occurrence of high procalcitonin concentrations in the case of sepsis and systemic infections may not be an isolated phenomenon but that in a similar manner elevated concentrations of other prohormones might also be measurable, so that the determination of such prohormones is a possible alternative to the procalcitonin determination or is suitable for supplementing the procalcitonin determination in individual cases or further confirming it in a diagnostically significant manner.

The discovery that it is not the complete procalcitonin

1-116 which is found in the serum of patients in the case of sepsis but a shortened procalcitonin 3-116 is finally also of potential interest for sepsis therapy. An article by Eric S. Nylen et al., Crit Care Med 1998, Vol. 26, No. 6, pages 1001-1006 describes experimental findings which indicate that the procalcitonin occurring in the case of sepsis is not only a diagnostically important marker which is formed, for example, as a metabolic waste product but appears to play an active role as a mediator in an inflammation process caused by infection, by virtue of the fact that procalcitonin can maintain and intensify inflammatory reaction. This role of procalcitonin is at present the subject of controversy, and the test results disclosed do not give a concurring picture.

The above-mentioned discovery that a procalcitonin shortened at the amino terminus by two amino acids occurs in the case of sepsis suggests that the procalcitonin which plays an active role in the case of sepsis and other inflammatory systemic infections is likely to be this shortened procalcitonin 3-116, and that studies carried out with the procalcitonin peptide of full length gave different or contradictory results, inter alia for this reason. It is well known that many physiologically active peptides are converted into their actual active form by cleavage, for example an initial elimination of a short peptide residue. A known example is angiotensin in which peptides having considerably different physiological activities are formed from the inactive angiotensinogen having 14 amino acids by successive elimination first of a tetrapeptide and then of a dipeptide and finally of an individual amino acid. The fact that relatively slight modifications of the N-terminus of the physiologically active peptide play a role in the immunological process and can lead to considerable changes in activity in the corresponding

peptides has been confirmed by a number of very recent publications, in which however no reference to septic pathological processes is made (cf. for example J Immunol 1998, Sept. 15, 161(6):2672-5; Biochemistry 1998, Sept. 8, 37(36): 12672-80; FEBS Lett 1998, July 31, 432 (1-2):73-6; J Biol Chem 1998, March 27; 273 (13):7222-7; J Exp Med 1997, Dec 1; 186(11):1865-72).

If it is assumed that procalcitonin 3-116 is actively involved in an inflammatory process and that specific molecular receptors or similar specific binders exist for this shortened procalcitonin, novel therapeutic possibilities are opened up for influencing the course of a sepsis with the use of procalcitonin 3-116 or of agonists and antagonists which interact with the receptors for the procalcitonin 3-116 and can thus influence the physiological reaction triggered by it and hence also an inflammatory process. The use of specific binders of procalcitonin 3-116, e.g. selective antibodies, is also a therapeutic approach which is opened up by the discoveries communicated herein.

Finally, that the dipeptidyl-aminopeptidase IV might play a role in the generation of procalcitonin 3-116 in the case of sepsis and systemic infections led to a further hypothesis, namely that it might also be possible to influence a sepsis or a sepsis-like inflammatory process therapeutically by influencing the activity of the dipeptidyl-aminopeptidase IV by blocking it, for example, by suitable selective binders, antibodies or similar receptor molecules.

It is the object of the present Patent Application to protect under patent law the novel technical teachings arising from the above novel discoveries and conclusions derived therefrom, to the extent that these are accessible to patent protection taking into account the

present state of knowledge.

The attached Patent Claims provisionally summarize such protectable teachings. Further protectable teachings may arise for a person skilled in the art from the complete text of the present application taking into account the experimental conditions and experimental results mentioned in the experimental section and the associated explanations. Rights are expressly reserved with regard to the claiming of such teachings by additional claims.

Selected experimental material which backs up the novel discovery or which demonstrates the correctness of the assumptions derived therefrom is presented below with reference to several diagrams.

In the Figures:

Figure 1 shows the results of a procalcitonin isolation and purification by HPLC from a pooled serum of collected sera from various patients with severe sepsis;

Figure 2 shows the results of a mass spectroscopic analysis of those fractions of the pooled serum from Fig. 1 which have a high procalcitonin immunoreactivity;

Figure 3 shows the results of the determination of the enzyme activity of dipeptidyl-aminopeptidase IV in septic sera and normal sera;

Figure 4 shows the results of the determination of procalcitonin in septic sera and normal sera in comparison with results of the determination of a further prohormone, namely pro-gastrin-releasing peptide (proGRP), in the same sera;

Figure 5 shows the results of the determination of procalcitonin in sera of a group of 20 normal persons and, on the other hand, 20 patients suffering from sepsis;

Figure 6 shows the results of the determination of pro-ANF (in pg/tube) in the same groups of normal persons and patients suffering from sepsis as in Figure 5;

Figure 7 shows the results of the determination of Pro-ADM (in pg/tube) in the same groups of normal persons and patients suffering from sepsis as in Figure 5;

Figure 8 shows the results of the determination of Pro-END (in pg/tube) in the same groups of normal persons and patients suffering from sepsis as in Figure 5; and

Figure 9 shows the results of the determination of Pro-BNP (in pg/tube) in the same groups of normal persons and patients suffering from sepsis as in Figure 5.

EXPERIMENTAL SECTION

A. Isolation and characterization of the endogenous procalcitonin peptide from sera of septic patients

By mixing serum samples from different patients suffering from severe sepsis, a mixed serum having a total volume of 68 ml was prepared. The procalcitonin concentration in the pooled serum obtained was determined with the aid of a commercial procalcitonin assay (LUMItest PCT, B.R.A.H.M.S. Diagnostica) as 280 ng/ml (total amount 19 µg). The pooled serum was mixed with an identical

volume of a buffer (68 ml; 10 mM EDTA, 1 mg/ml mouse-IgG, 2 mg/ml sheep-IgG, 2 mg/ml bovine IgG, 0.1 mmol leupeptin, 50 μM Amastatin in PBS) and the procalcitonin contained in the sample was isolated and purified by affinity chromatography.

For this purpose, the total pooled sample was pumped at a flow rate of 0.5 ml/min four times in succession over an affinity column (0.5 x 1 cm, anti-calcitonin antibodies, bound to Carbolink from Pierce, procalcitonin binding capacity about 20 μg). The column was then washed with 30 ml of PBS, and the bound peptide was eluted with the aid of 50 mM acetic acid (pH about 2.0). The column outflow was monitored continuously for absorption at 280 nm, and the protein fraction eluted by the acetic acid was collected (final volume 2.0 ml).

The material collected in this manner was purified by reversed-phase HPLC over an rpC_{18} column μ Bondapak 0.4 x 30 mm (from Waters). The flow rate was 1 ml/min, and the mobile phase and elution conditions are shown in Table 1 below.

Table 1: Elution conditions for rp-HPLC of procalcitonin

Mobile phase A:	5% acetonitrile 20 mM NH_4 acetate		
Mobile phase B:	90% acetonitrile 20 mM NH_4 acetate		
Gradient:	0.0 min	100% A	0% B
	2.5 min	100% A	0% B
	5.0 min	89% A	11% B
	55.0 min	56% A	44% B
	60.0 min	0% A	100% B

The column outflow was measured continuously for its

absorption at 214 nm and collected in fractions of 0.25 ml. With the aid of a commercial procalcitonin assay (LUMItest PCT, B.R.A.H.M.S Diagnostica) those fractions in which a PCT immunoreactivity was detectable were determined. It was found that the main immunoreactivity was eluted in the 51st fraction as a sharp band. In addition, protein fractions having a heterogeneous composition and lower PCT immunoreactivities were obtained in fractions 39 to 49.

Figure 1 shows the PCT immunoreactivity (expressed as ng PCT/ml) determined for the individual collected fractions of the rp HPLC chromatography, superposed with a curve which shows the optical density (OD) of the eluted fractions.

All fractions which had a positive procalcitonin immunoreactivity were dried with nitrogen by gassing. Thereafter, the samples were analyzed by mass spectrometry and subjected to an N-terminal sequencing.

In the mass spectrometric analysis (MALDI-TOF method), the profile shown in Figure 2 was obtained for fractions 50-52, from which profile a molar mass of 12640 ± 15 resulted. All other fractions (36-49, 53-59) investigated by mass spectrometry showed heterogeneous mass distributions with molar masses <12640. Their individual mass gave an intensity of <2% in comparison with the intensity of the mass of the fractions 50-52. It was thus demonstrated that the procalcitonin immunoreactivity in sera of patients suffering from sepsis is associated with a mass of 12640 ± 15. None of the fractions obtained had a higher mass.

The peptides contained in fractions 36-59 were subjected to an N-terminal sequencing. Here too, the content of fractions 36-49 and 53-59 proved to be heterogeneous,

i.e. a multiplicity of N-termini was determined.

For the fractions 50-52, in which the predominant procalcitonin immunoreactivity was to be found, it emerged that the peptides contained therein clearly have the following N-terminus (15 amino acids):

Phe Arg Ser Ala Leu Glu Ser Ser Pro Ala Asp Pro Ala Thr Leu

The peptide from fractions 50-52 was then digested by means of protease Glu-C or trypsin, and the resulting fragments were recovered in a manner known per se by means of SMART-HPLC and then investigated by mass spectrometry and sequence analysis.

A sequence which corresponded completely with the sequence of the amino acids 3-116 of the known procalcitonin 1-116 was obtained. The theoretical mass of the sequence was 12627, which is in agreement with the mass of 12640 ± 15 determined by mass spectrometry.

Consequently, it was demonstrated that a procalcitonin peptide which comprises 114 amino acids and is to be designated as procalcitonin 3-116 circulates in the blood of patients suffering from sepsis. The peptide is not changed by posttranslational modifications, such as phosphorylations or glycosylations.

The procalcitonin 3-116 has not yet been discussed to date in the scientific literature as a possible endogenous procalcitonin partial peptide, and there has therefore also been no reason to date for a person skilled in the art specifically to prepare this peptide and to investigate it with regard to its properties. However, the above findings have now provided a reason for the specific preparation of said procalcitonin 3-116 by genetic engineering techniques. Its preparation is

described below.

B. Cloning, expression and purification of recombinant procalcitonin 3-116

1. Cloning

The DNA fragments coding for procalcitonin 3-116 (abbreviated below to PCT 114) were isolated from a human thyroid cDNA pool using PCR amplification with the aid of suitable oligonucleotide primer. The desired fragment was cloned by means of conventional methods (Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K (1991), Current Protocols in Molecular Biology, John Wiley & Sons, New York), and the correct nucleotide sequence was verified by DNA sequencing and comparison with the known DNA sequence coding for procalcitonin.

For the expression of the cDNA coding for PCT 114, a vector was used which contains, in addition to a T7 promoter, a region which codes for the signal peptide of the so-called pelB protein. This pelB signal peptide ensures that a fusion protein formed after cloning and expression is transported through the cytoplasmic membrane of the host cells used for the expression into the periplasmic space. During this transport process, the N-terminal signal peptide is simultaneously separated by a signal peptidase located on the membrane (Stader JA and Silhavy TJ (1990), Engineering Escherichia coli to secrete heterologous gene products, Methods Enzymol. 185, 166-187). This procedure guarantees that the expression product found has exactly the desired sequence. In this procedure, the N-terminal methionine required in other expression methods is absent.

After the cloning of the cDNA for PCT 114 into a vector of said type and transformation of E. coli with this

vector, procalcitonin 3-116 was expressed. The periplasmic fraction with the expressed procalcitonin 3-116 was isolated in a manner known per se (Neu HC and Heppel LA (1965), The release of enzymes from Escherichia coli by osmotic shock and during the formation of spheroblasts, J. Biol. Chem. 240, 3685-3692). After centrifuging (100,000 g, 30 min, 4°C) and filtration of the liquid supernatant (0.2 μm) the filtrate obtained was separated by anion exchange chromatography. The fractions with procalcitonin immunoreactivity were combined and were purified by reversed-phase HPLC, as described in connection with the isolation of PCT 3-116 from septic sera.

All fractions with procalcitonin immunoreactivity were combined and lyophilized. As shown by checking the material by means of SDS-PAGE, the material thus obtained was at least 95% pure.

The identity of the expressed and purified peptide as procalcitonin 3-116 was confirmed by mass spectrometry and sequence analysis.

The recombinant procalcitonin 3-116 obtained is a novel recombinant peptide and can be used in this form for the preparation of immune reagents and investigated with respect to suitability as a therapeutic or with respect to its ability, in the context of the above-mentioned publication (Eric S Nylen, loc. cit.), to have prophylactic and therapeutic activity.

For the preparation of calibrators for PCT assays, the method described above for the preparation of procalcitonin 3-116 by genetic engineering was used in essentially identical form also for the preparation of the complete procalcitonin 1-116 and of procalcitonin 2-116.

C. Determination of the dipeptidyl-aminopeptidase IV (DAP IV) activity in normal human sera and sera from patients with severe sepsis

20 serum samples each from healthy normal persons and from patients suffering from sepsis were investigated with respect to their dipeptidyl-aminopeptidase IV-specific enzyme activity. The DAP IV enzyme activity was measured fluorometrically in a manner known per se using Lys-Pro-4-methoxy-beta-naphthylamide. For this purpose, in each case 2 μl of the serum to be tested with 3 ml of substrate (50 μg/ml Lys-Pro-4-methoxy-beta-naphthylamide, 50 mM Tris/HCl, pH 7.5) and the resulting fluorescence was measured continuously at an emission wavelength of 410 nm with excitation with light having a wavelength of 340 nm. The fluorescence signal was calibrated by means of a 4-methoxynaphthylamine solution. The enzyme activity determined in this manner is stated in nmol/min.

The results obtained are shown in Figure 3.

It is clear that the DAP IV enzyme activity in the sepsis sera is substantially lower than that in the sera of healthy normal persons (blood donor sera). Thus, the determination of the DAP IV enzyme activity in plasma or serum can also be used for detecting sepsis in patients sera.

The substantially reduced plasma concentration of DAP IV in the case of sepsis may be regarded on the one hand as evidence that DAP IV is involved in a sepsis process. On the other hand, the results indicate that it cannot be the concentration of DAP IV in the plasma that is responsible for the formation of procalcitonin 3-116. Rather, the results obtained suggest the conclusion that procalcitonin 3-116 is formed by tissue- or cell-bound DAP IV, possibly intracellularly, and is liberated from

procalcitonin-producing or procalcitonin-storing cells.

The information contained in the literature to the effect that DAP IV is expressed from activated T-cells (cf. Hegen and Oravecz, Protein Reviews on the WEB; Fleischer, loc. cit.) indicates a close relationship between the expression of DAP IV and the activity state of the immune system, which, in the case of a septic systemic infection, is under extreme stress and therefore exhibits typical reactions which manifest themselves, inter alia, in greatly increased procalcitonin 3-116 formation.

Apart from the possibilities arising out of the above findings, for determining DAP IV in the course of the sepsis diagnosis, the above results may also indicate that the processes taking place in a cascade-like manner during a sepsis can be influenced therapeutically by DAP IV inhibitors, so that it might be possible to prevent or to reduce the liberation of procalcitonin 3-116 and other hormones or converted prohormones under sepsis, enabling pathological consequences for this prohormone liberation to be reduced or avoided.

D. Determination of the concentrations of prohormones other than procalcitonin in the case of sepsis

The fact that it is not the complete prohormone procalcitonin which is released in the case of sepsis, but a modified prohormone shortened by an Xaa-Pro dipeptide, led to the hypothesis that not only is procalcitonin 3-116 liberated in the case of sepsis but procalcitonin 3-116 is perhaps only one representative of a whole group of prohormones or similar peptides, for example those having immunomodulatory properties, which are liberated to a high degree and possibly in converted form in the case of sepsis.

Checking of known prohormones and of the amino acid sequences stated in the literature for these prohormones showed that in actual fact most known prohormones have at the amino terminus a dipeptide which can be defined as Xaa-Pro and which can therefore be eliminated in a sense similar to that observed in the case of procalcitonin. Specifically, dipeptides of said type are present at the amino terminus of a very large number of prohormones or immunomodulators. The following list of literature data in the form of a table gives an overview of some selected prohormones or immunomodulators, the dipeptide to be found in these at the amino terminus and their total number of amino acids.

The prohormones shown in Table 2 are examples of prohormones whose concentrations may be elevated in the case of sepsis, although the list is not to be regarded as exhaustive. In the case of the immunomodulators, elimination of a dipeptide is likely to influence the activity.

Table 2:

Prohormone/ Immunomodulator	Dipeptide at the N-terminus	Total number of all amino acids
pro-Endothelin-1 (pro-END)	Ala Pro	195
pro-Brain-natriuretic peptide (pro-BNP)	His Pro	108
Pro-Atrial-natriuretic peptide (pro-ANP; also pro-atrionatriuretic factor, pro-ANF)	Asn Pro	128
pro-Leptin	Val Pro	146
pro-Neuropeptide Y	Tyr Pro	69
pro-Somatostatin	Ala Pro	92
pro-Neuropeptide YY	Thr Pro	69
Interleukin 6	Val Pro	183
Interleukin 10	Ser Pro	160
pro-Gastrin-releasing peptide (proGRP)	Val Pro	115
pro-Opiomelanocortin	Trp Cys	241
pro-Adrenomedullin (pro-ADM)	Ala Arg	164
Procalcitonin (PCT)	Ala Pro	116

The experimental findings to date actually indicate that, in the case of a systemic infection, such as sepsis, in general prohormones and peptide immunomodulators, such as interleukins, are possibly liberated with modification by elimination of dipeptides at the amino terminus and that these possibly initiate further subsequent steps in the cascade of an immune response by interaction with associated specific receptors or other binders.

Parallel with a procalcitonin determination in normal sera and sera from patients suffering from sepsis, the determination of further prohormones, which had been

chosen fairly randomly, was also carried out. These were (i) pro-gastrin-releasing peptide (proGRP), (ii) pro-atrial-natriuretic peptide (pro-ANP or pro-ANF), (iii) pro-adrenomedullin (pro-ADM), (iv) pro-endothelin (pro-END) and (v) pro-brain-natriuretic peptide (pro-BNP).

D.1. Determination of proGRP in sera from patients suffering from sepsis and from normal persons

An assay is commercially available for the determination of proGRP. In a recent publication, proGRP is described as a tumour marker in small-cell bronchial carcinoma (Petra Stieber et al., J Lab Med 1997, 21(6):336-344). The assay for the determination of proGRP is commercially available from the Tonen Corporation under the name ProGRP ELISA KIT™.

Using this kit and following the procedure prescribed in the information for use of the commercial kit, the measured results shown in Figure 4 were obtained.

A comparison of the values obtained for procalcitonin on the one hand and proGRP on the other hand shows that the distinction between normal sera and sepsis sera is clearer in the case of procalcitonin but that the proGRP concentrations are elevated in a manner substantially similar to those of procalcitonin.

D.2. Determination of pro-ANF, pro-ADM, pro-END and pro-BNP in sera from patients suffering from sepsis and from normal persons

For the determination of the (pre)prohormones pro-ANF, pro-ADM, pro-END and pro-BNP, assays are commercially available in kit form from DRG (DRG Instruments GmbH, D-35018 Marburg, Germany) and were used for the following measurements in accordance with the manufacturer's

instructions.

Specifically, the following were used:

For the determination of pro-ANF, the Prepro-ANF 26-55 (human) RIA kit; for the determination of pro-ADM, the Pro-Adrenomedullin 45-92 (human) RIA kit; for the determination of pro-END, the Prepro-Endothelin 18-50 (human) RIA kit; and for the determination of Pro-BNP, the Prepro-BNP 22-46 (human) RIA kit from the above-mentioned company DRG.

In the sera of a group of 20 normal persons A-T and of 20 patients suffering from sepsis, the above-mentioned prohormones and, parallel to these, procalcitonin were determined. The results are summarized in Table 3 below. The data in Table 3 are also shown graphically in Figures 5 to 9.

The results shown show a more or less clear increase in the values for all measured prohormones in the case of patients suffering from sepsis compared with normal persons, although the distinction between normal persons and patients suffering from sepsis is most pronounced in the determination of procalcitonin.

The supplementary literature search furthermore reveals a publication in J. Endocr. (1988) 119, pages 159-165 which was concerned with characterization of pro-Opiomelanocortin (POMC)-related peptides in septic shock. The publication considers the question of increased endogenous opioid activity in septic shock and the influencing of selectivity by administration of steroids. A direct effect of an infectious process or the influencing of the measured values by antibiotics is not discussed. On the basis of the problem in said publication, there is no logical possibility of a

generalized discussion of the reported results including other prohormone peptides. It is only in view of the teaching of the present invention, disclosed herein, that said publication can be interpreted retrospectively as a further indication of a general increase in prohormones in the case of sepsis.

Testing for further prohormones is to be regarded as a routine measure in view of the results disclosed herein and, if such tests lead to positive results and the determination of such further prohormones is used for the diagnosis of sepsis, use will therefore have been made of the teaching of the present Application.

Table 3:

	PCT [ng/ml]	Pro-ANF [pg/ tube]	Pro-ADM [pg/ tube]	Pro-END [pg/ tube]	Pro-BNP [pg/ tube]
Normal patients:					
A	0.07	69.4	64	29.3	31.1
B	0.26	26.6	50.2	15.4	30.1
C	0.10	14.7	1.0	1.0	24.7
D	0.06	53.2	77.8	19.3	27.4
E	0.06	51.1	66.5	20.6	25.4
F	0.04	95.5	53.5	28.2	28.9
G	0.07	117	83.5	18.3	17.3
H	0.10	88.1	52.3	25.1	28.1
I	0.10	69.4	107	23.2	25
J	0.07	38.3	91.1	26.1	25.7
K	0.06	111	64.9	22.8	29.6
L	0.09	73.8	66.3	32.4	21.6
M	0.07	42	58.9	27.1	23.7
N	0.09	107	114	27.3	31.2
O	0.10	56.7	62.5	20.3	26.2
P	0.10	47.2	51.7	24.5	33.3
Q	0.12	155	72.5	34.6	36.7
R	0.13	92.1	64.7	29.6	35.2
S	0.12	153	100	34.7	36
T	0.11	78	69.6	27.1	37.7
Sepsis patients:					
13a	1.1	333	195	70	34.9
10a	11.9	62.5	154	30.5	30.4
18b	6.4	323	209	75.4	46.4
19b	21.2	346	180	71.6	50.7
8a	1.8	303	203	70.3	48.8
9a	24.7	271	205	78.4	33.6
9b	29.0	305	210	62.5	40.3
12b	5.8	324	204	67.4	36.1
10b	1.9	127	128	30.5	33.9
16a	86.7	347	198	83.3	39
8b	1.1	138	153	29.6	33.7
20a	1.4	167	176	41.3	30.9
20b	1.1	170	178	39	34.9
13b	0.8	295	186	45.3	36.9
19a	17.6	354	201	58.6	54.5
7b	2.5	356	199	78.6	
7a	2.9	345	197	148	
16b	40.0	343	197	88.1	43.9
12a	5.2	327	216	76.1	34.3
15b	1.9	420	215	82.5	52

Patent Claims

1. Method for the differential-diagnostic early detection and detection, for the assessment of the severity, and for the assessment of the success of a therapeutic treatment of sepsis and severe infections, in particular sepsis-like systemic infections, characterized in that the content of at least one peptide prohormone other than procalcitonin and/or of a partial peptide derived therefrom, which is not the mature hormone obtainable from said peptide prohormone, is determined in a sample of a biological fluid of a patient, and the presence of a sepsis or sepsis-like systemic infection, its severity and/or the success of a therapeutic treatment are determined from the detected presence and/or amount of the determined peptide prohormone.

2. Method according to Claim 1, characterized in that the peptide prohormone is selected from the group consisting of pro-gastric-releasing peptide (proGRP), pro-endothelin-1 (pro-END), pro-brain-natriuretic peptide (pro-BNP), pro-atrial-natriuretic peptide (pro-ANP or pro-ANF), pro-leptin, pro-neuropeptide-Y, pro-somatostatin, pro-neuropeptide-YY or pro-adrenomedullin (pro-ADM).

3. Method according to either of Claims 1 and 2, characterized in that by the determination a partial peptide is detected which differs from the known complete peptide prohormone by the lack of a dipeptide at the amino terminus thereof, as it can be cleaved off by dipeptidyl-aminopeptidase IV (DP IV or DAP IV or CD26) from the end of a peptide.

4. Method according to Claim 3, characterized in that

the dipeptide is an Xaa-Pro dipeptide, Xaa representing the amino-terminal amino acid of the complete prohormone peptide.

5. Method according to any of Claims 1 to 4, characterized in that said determination of said peptide prohormone is carried out as an immunoassay or precipitation assay, and a diagnosis of the presence of sepsis or severe sepsis-like infections is made if the concentration of the peptide prohormone determined is significantly higher than the values for the same prohormone observed in healthy normal persons.

6. Method for the differential-diagnostic early detection, for the detection, and for the assessment of the severity and for the assessment of the success of a therapeutic treatment of a sepsis and sepsis-like systemic infections, characterized in that the content of dipeptidyl-peptidase IV (DP IV; dipeptidyl-aminopeptidase IV; DAP IV or CD26) is determined in a serum or plasma sample of a patient and the presence of a sepsis or sepsis-like systemic infection is diagnosed on the basis of a concentration of dipeptidyl-peptidase IV which is significantly reduced compared with healthy normal subjects.

7. Procalcitonin 3-116 prepared by genetic engineering.

8. Method for the preparation of procalcitonin 3-116 by genetic engineering, comprising

- inserting a cDNA sequence coding for the 114 amino acids of procalcitonin 3-116 into a suitable vector,
- transforming suitable host cells with the vector formed so that they express procalcitonin 3-116,

- working up said host cells,
- recovering a fraction containing the expressed procalcitonin 3-116, and
- obtaining from said fraction said procalcitonin 3-116 as a product prepared by genetic engineering in at least 90% purity by chromatographic purification.

9. Use of recombinant procalcitonin 3-116 as a calibrator in procalcitonin assays or for the preparation of therapeutics for the prevention and treatment of sepsis and sepsis-like systemic infections.

10. Method for the measurement of procalcitonin 3-116 as an indication-independent diagnostic parameter.

Abstract

Methods and substances for the diagnosis and therapy of sepsis and sepsis-like systemic infections

Uses of recombinant procalcitonin 3-116 in the diagnosis and therapy of septic diseases and the measurement of prohormones other than procalcitonin, and of dipeptidyl peptidase IV, as biomarkers in the diagnosis of sepsis.

HPLC-Reinigung von affinitätsgereinigtem humanen PCT

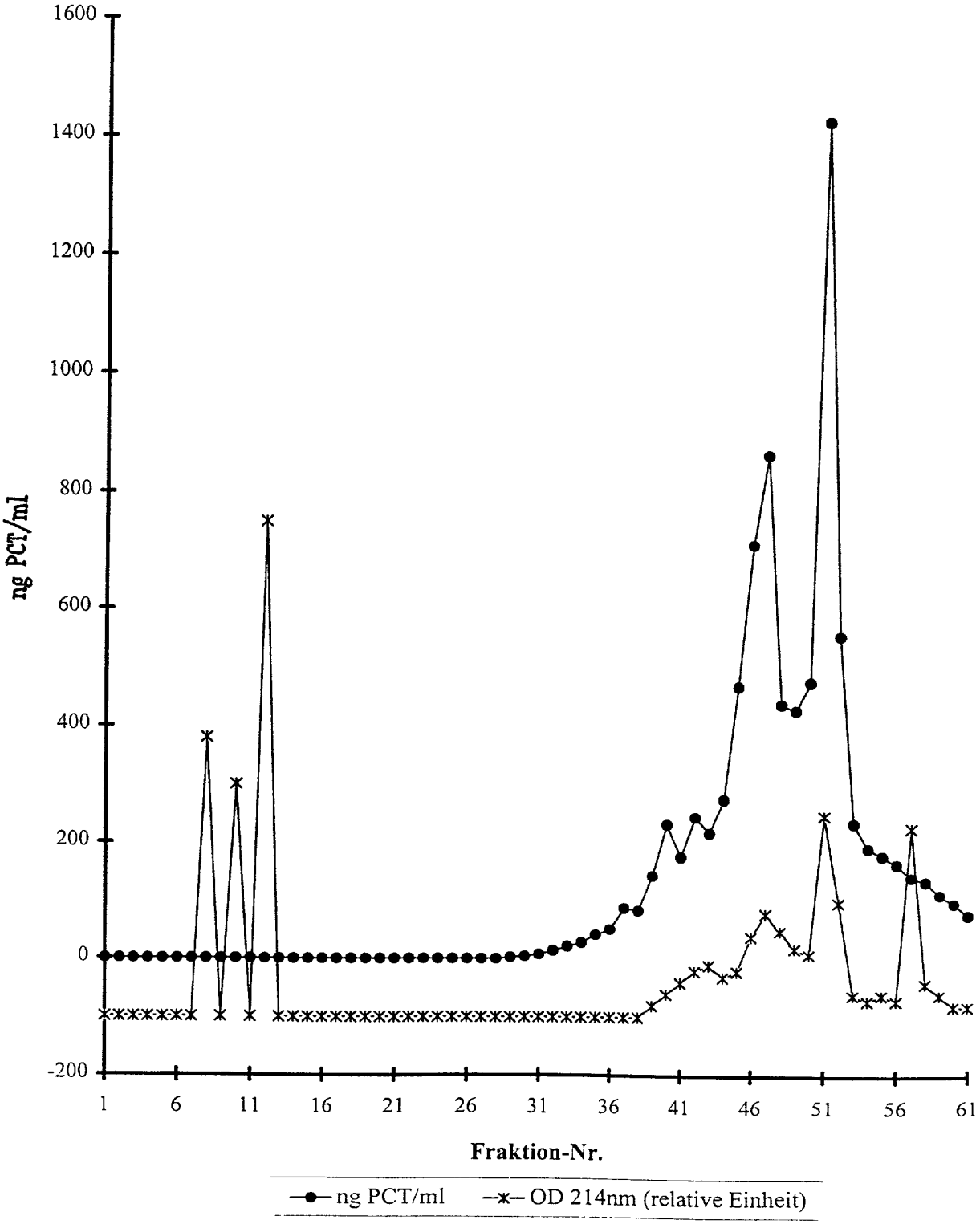
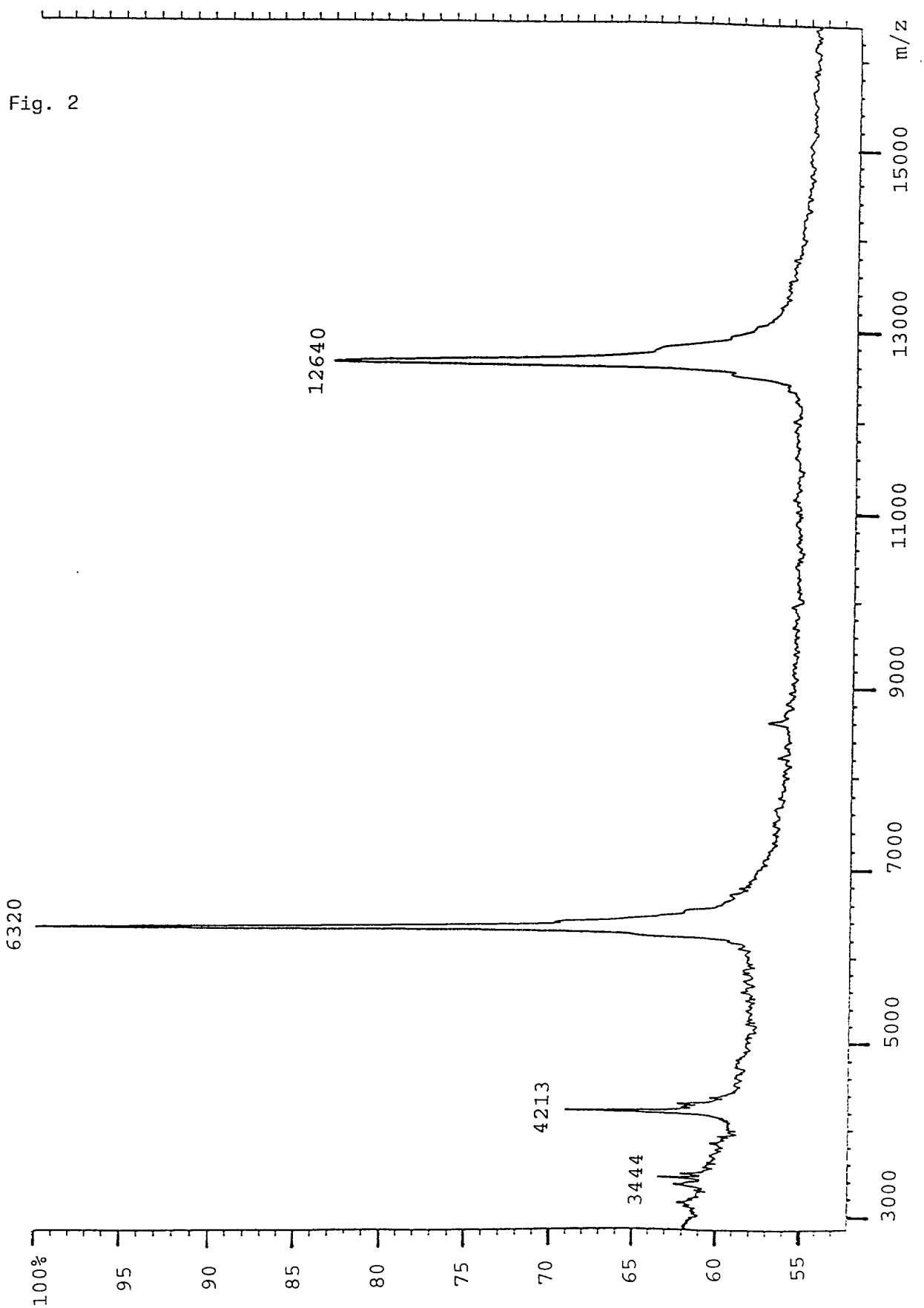


Fig. 1

Fig. 2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90
85
80
75
70
65
60
55

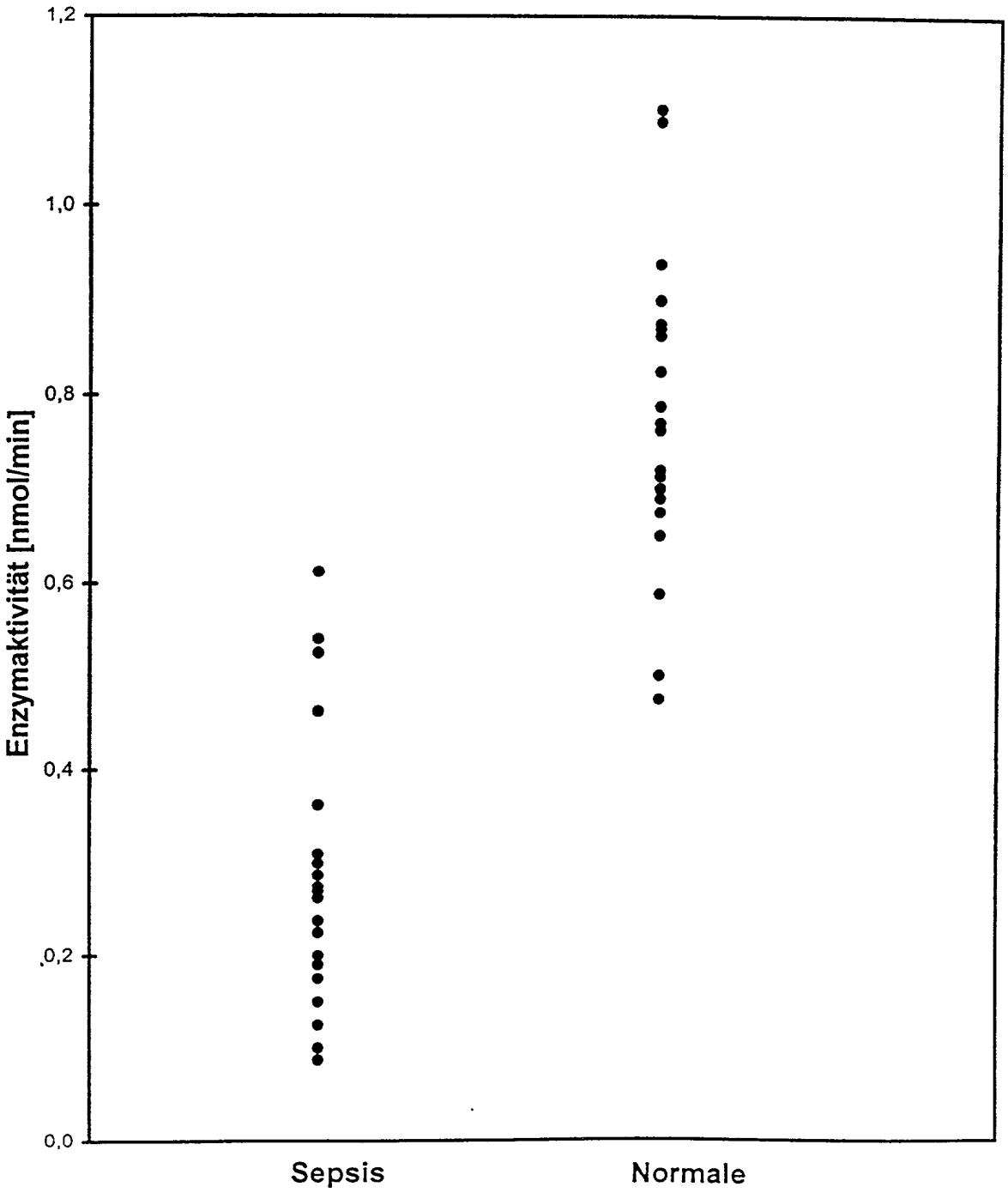

Enzymaktivität von DAP IV in septischen Seren vs. Blutspenderseren
Enzymaktivität [nmol/min]
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Sepsis
Normale

Fig. 3

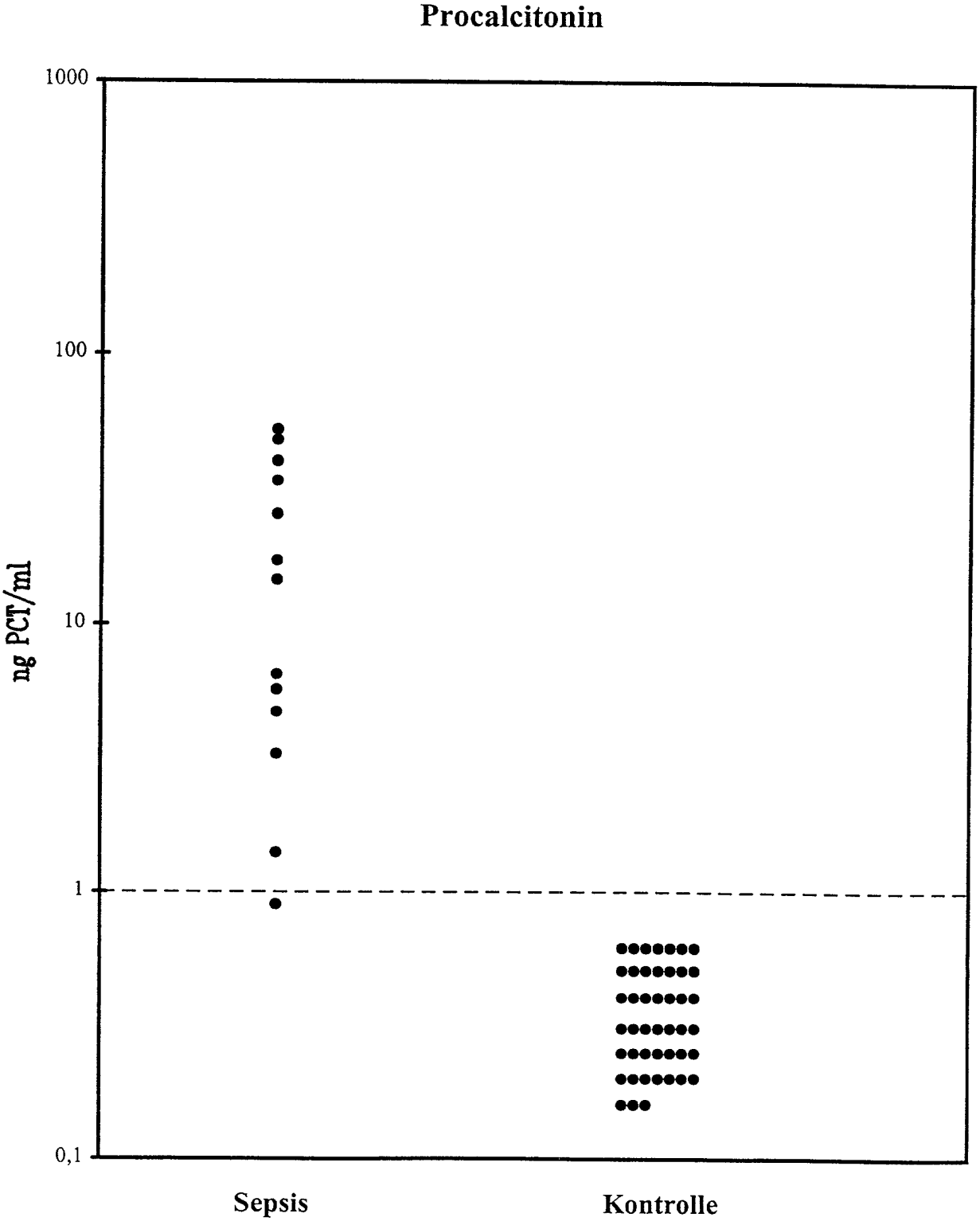


Fig. 4a

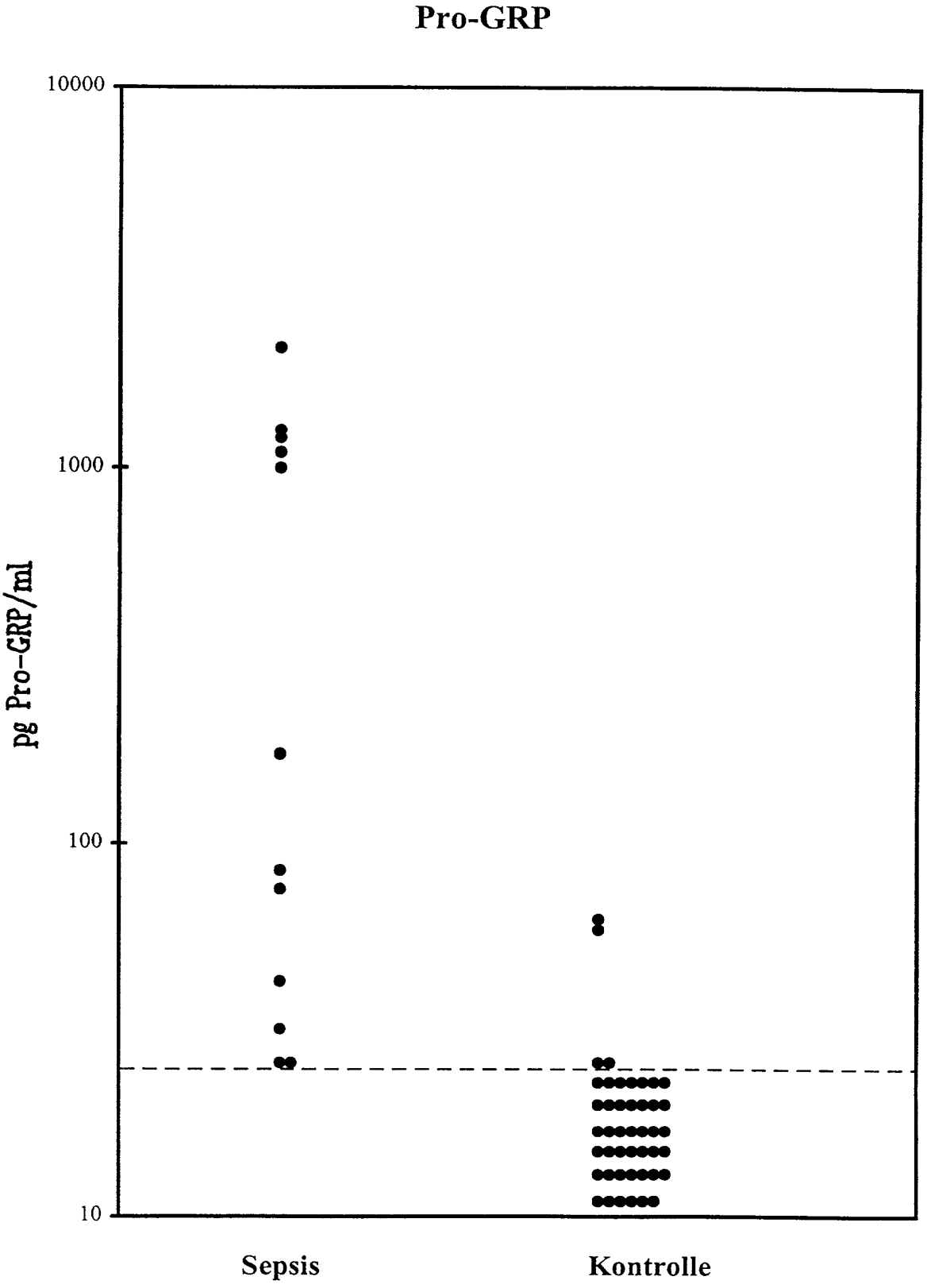


Fig. 4b

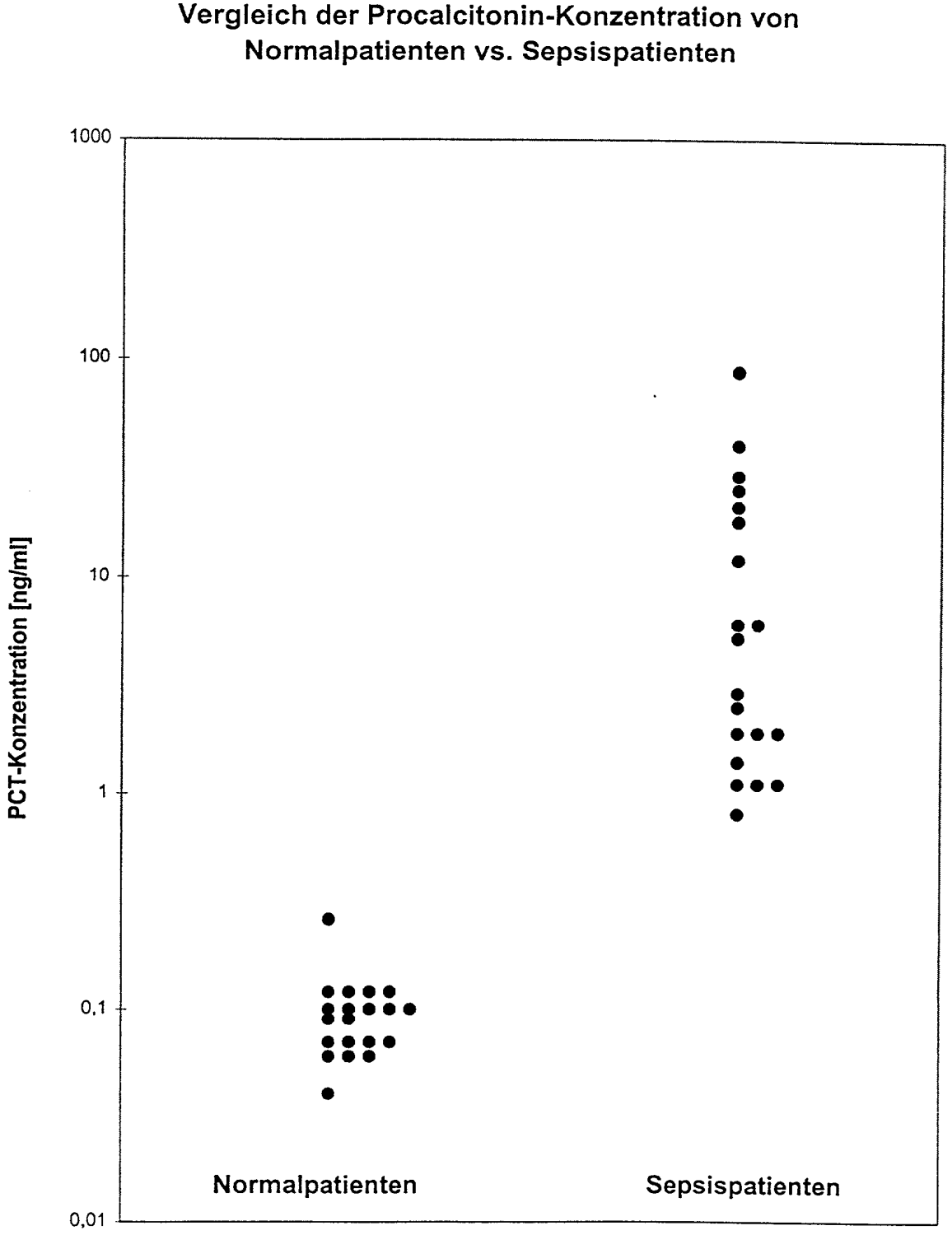

Vergleich der Procalcitonin-Konzentration von Normalpatienten vs. Sepsispatienten
PCT-Konzentration [ng/ml]
1000
100
10
1
0,1
0,01
Normalpatienten
Sepsispatienten

Fig. 5

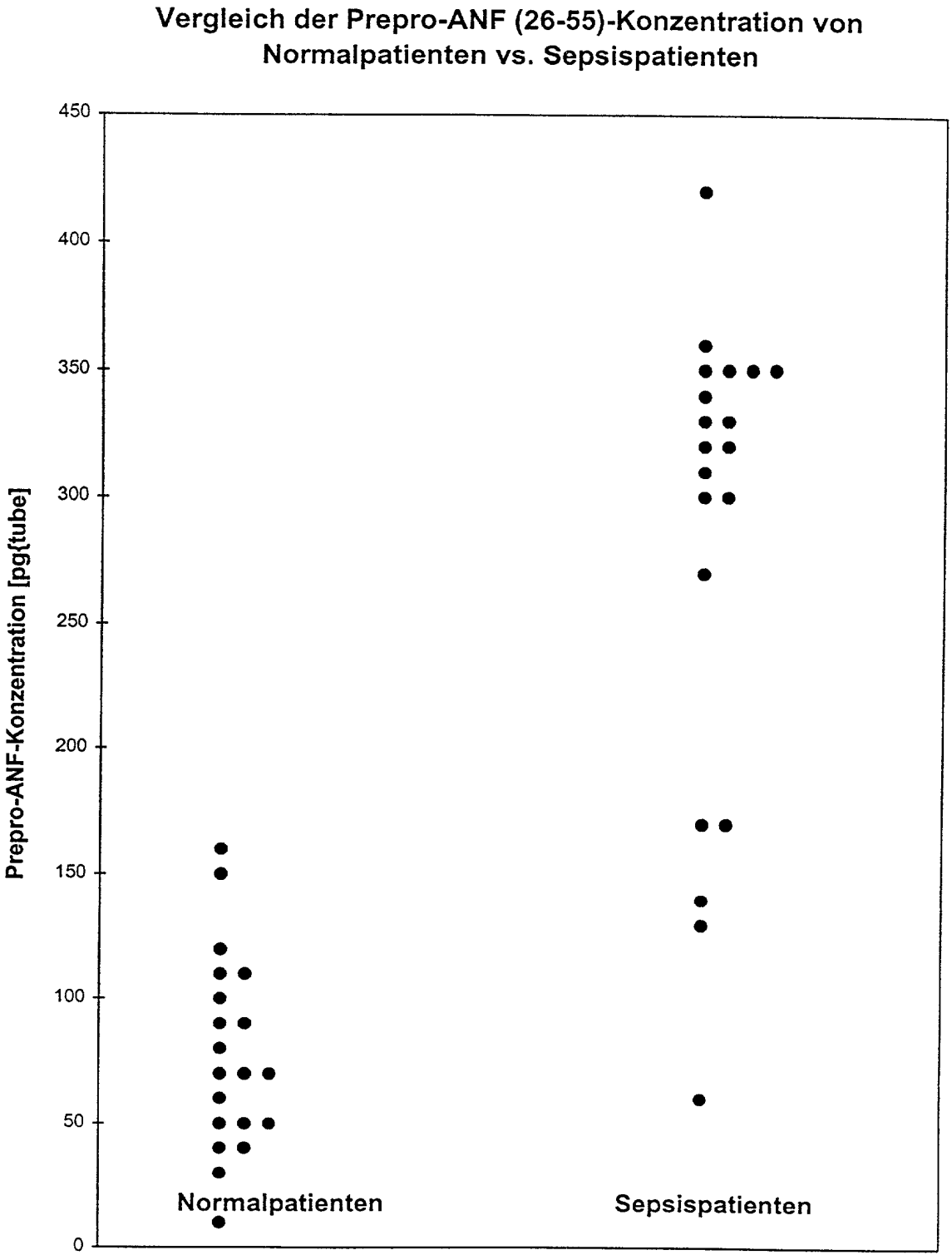

Vergleich der Prepro-ANF (26-55)-Konzentration von Normalpatienten vs. Sepsispatienten
Prepro-ANF-Konzentration [pg/tube]
450
400
350
300
250
200
150
100
50
0
Normalpatienten
Sepsispatienten

Fig. 6

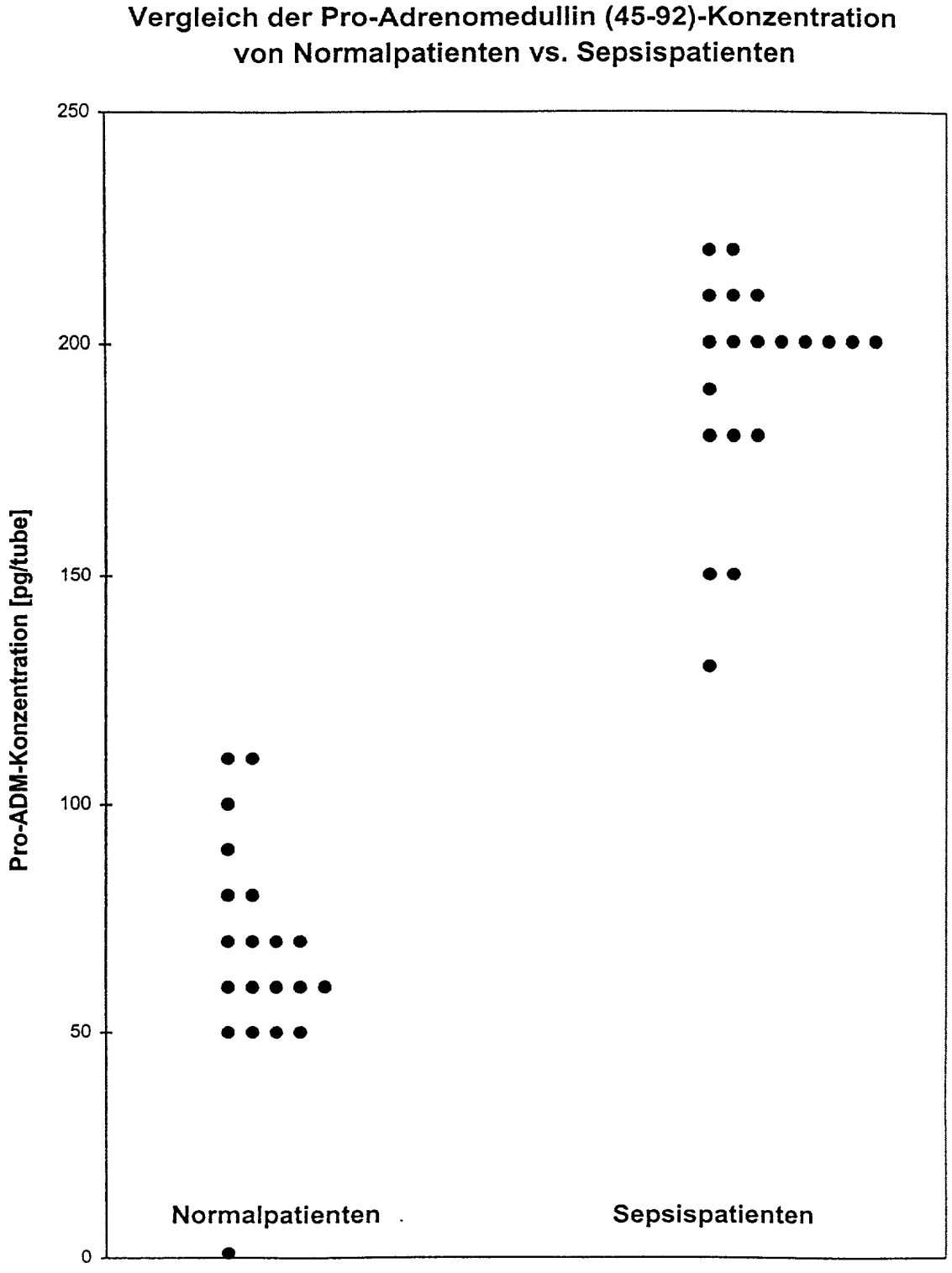

Vergleich der Pro-Adrenomedullin (45-92)-Konzentration
von Normalpatienten vs. Sepsispatienten
Pro-ADM-Konzentration [pg/tube]
250
200
150
100
50
0
Normalpatienten
Sepsispatienten

Fig. 7

Vergleich der Prepro-Endothelin (18-50)-Konzentration von Normalpatienten vs. Sepsispatienten

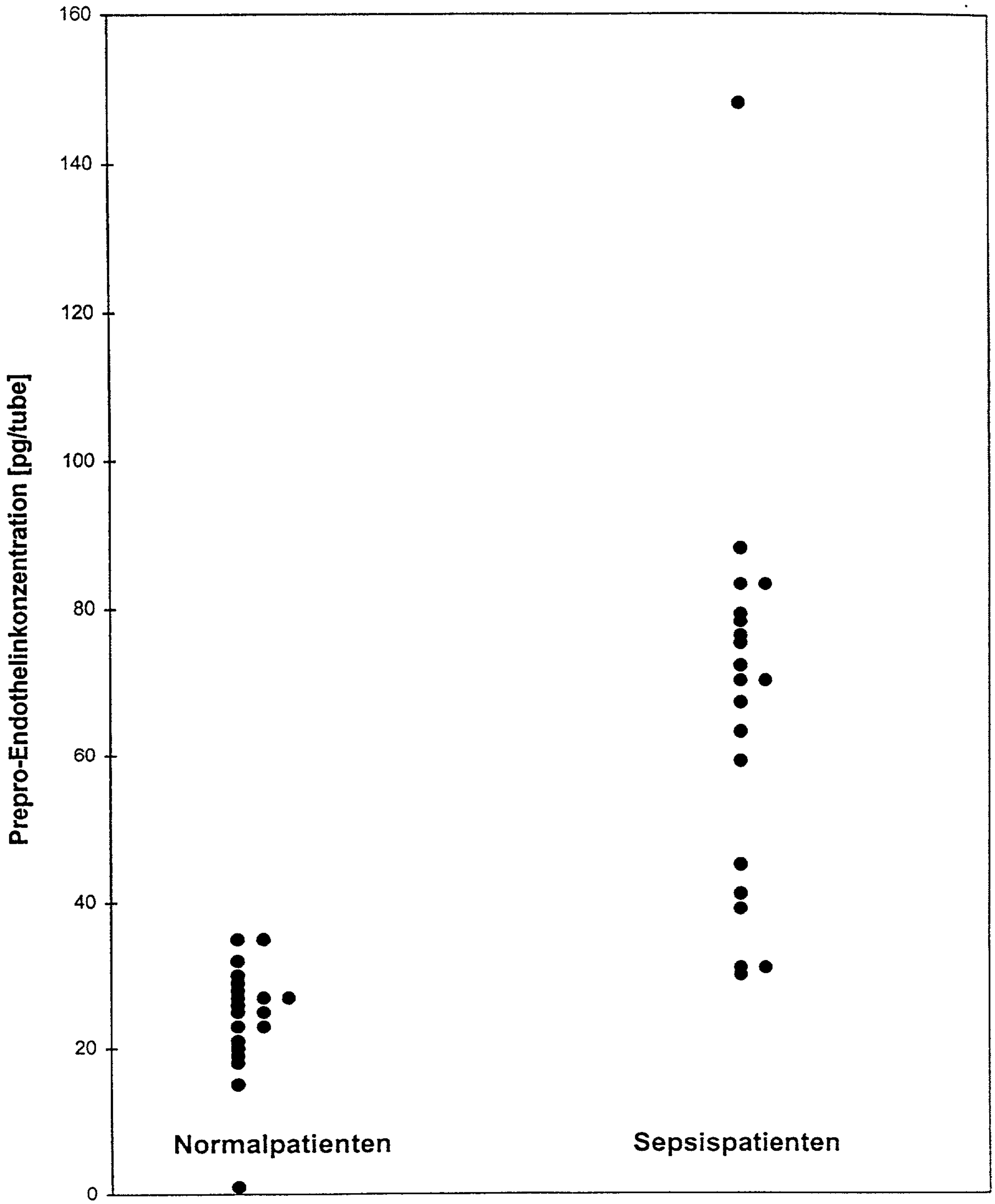


Fig. 8

Vergleich der Prepro-BNP (22-46)-Konzentration von Normalpatienten vs. Sepsispatienten

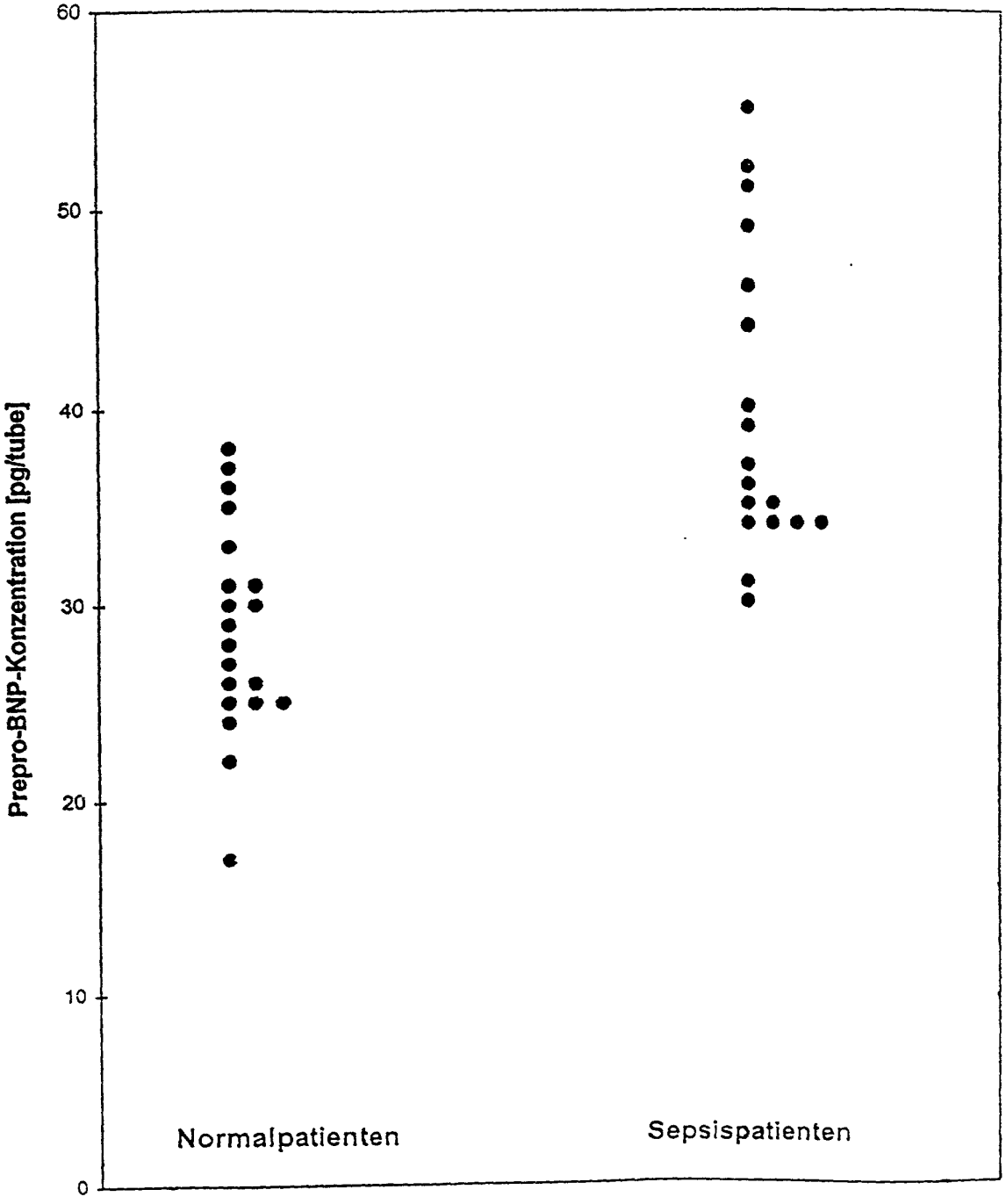


Fig. 9

FOR UTILITY/DESIGN CIP/PCT NATIONAL/PLANT ORIGINAL/SUBSTITUTE/SUPPLEMENTAL DECLARATIONS

RULE 63 (37 C.F.R. 1.63) DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PW FORM

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the **INVENTION ENTITLED METHOD AND SUBSTANCES FOR DIAGNOSIS AND THERAPY OF SEPSIS AND SEPSIS-LIKE SYSTEMIC INFECTIONS**

the specification of which (CHECK applicable BOX(ES))

X A. ☐ is attached hereto.

BOX(ES) → B. ☒ was filed on March 30, 2001 as U.S. Application No. 09/806,437

→ → C. ☒ was filed as PCT International Application No. PCT/ EP99/07692 on October 13, 1999

and (if applicable to U.S. or PCT application) was amended on

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. Except as noted below, I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International Application which designated at least one other country than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International Application, filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority claimed, before the filing date of this application:

PRIOR FOREIGN APPLICATION(S) Number	Country	Day/MONTH/Year Filed	Date first Laid-open or Published	Date Patented or Granted	Priority NOT Claimed
198 47 690.6	GERMANY	15 October 1998			

If more prior foreign applications, X box *at bottom* and continue on attached page.

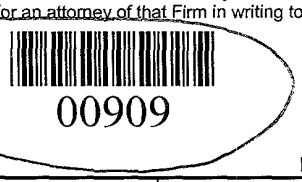
Except as noted below, I hereby claim domestic priority benefit under 35 U.S.C. 119(e) or 120 and/or 365(c) of the indicated United States applications listed below and PCT international applications listed above or below and, if this is a continuation-in-part (CIP) application, insofar as the subject matter disclosed and claimed in this application is in addition to that disclosed in such prior applications, I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56 which became available between the filing date of each such prior application and the national or PCT international filing date of this application:

PRIOR U.S. PROVISIONAL, NONPROVISIONAL AND/OR PCT APPLICATION(S) Application No. (series code/serial no.)	Day/MONTH/Year Filed	Status pending, abandoned, patented	Priority NOT Claimed

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint Pillsbury Winthrop LLP, Intellectual Property Group, telephone number (202) 861-3000 (to whom all communications are to be directed), and persons of that firm who are associated with USPTO Customer No. 909 (see below label) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to delete from that Customer No names of persons no longer with their firm, to add new persons of their Firm to that Customer No , and to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct the above Firm and/or an attorney of that Firm in writing to the contrary.

USE ONLY FOR PILLSBURY WINTHROP



1.00

(1) **INVENTOR'S SIGNATURE:** Date: 10/04/01

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2.00

(2) **INVENTOR'S SIGNATURE:** Date: 11.4.01

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	City	State/Foreign Country	Country of Citizenship
Mailing Address	Holsteinische Strasse 28, Berlin, Germany		
(include Zip Code)	D-12161		

☒ FOR ADDITIONAL INVENTORS see attached page.

☐ See additional foreign priorities on attached page (incorporated herein by reference).

Atty. Dkt. No. P 279277 (M#)

DECLARATION AND POWER OF ATTORNEY
(continued)
ADDITIONAL INVENTORS:

3-00

(3) **INVENTOR'S SIGNATURE:** Date: 11/04/01

	Wolfgang		**WEGLÖHNER**
	First	Middle Initial	Family Name
Residence	Berlin	GERMANY DE X	GERMANY
	City	State/Foreign Country	Country of Citizenship
Mailing Address	Lorenzweg 2, Berlin, Germany		
(include Zip Code)	D-12099		

(4) **INVENTOR'S SIGNATURE:** Date:

	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship
Mailing Address			
(include Zip Code)			

(5) **INVENTOR'S SIGNATURE:** Date:

	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship
Mailing Address			
(include Zip Code)			

(6) **INVENTOR'S SIGNATURE:** Date:

	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship
Mailing Address			
(include Zip Code)			

(7) **INVENTOR'S SIGNATURE:** Date:

	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship
Mailing Address			
(include Zip Code)			

(8) **INVENTOR'S SIGNATURE:** Date:

	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship
Mailing Address			
(include Zip Code)			

(9) **INVENTOR'S SIGNATURE:** Date:

	First	Middle Initial	Family Name
Residence			
	City	State/Foreign Country	Country of Citizenship
Mailing Address			
(include Zip Code)			